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An analysis in support of sediment quality thresholds for polycyclic aromatic hydrocarbons (PAHs) to protect estuarine fish

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ABSTRACT

1. Under the US Endangered Species Act and the Essential Fish Habitat provisions of the Sustainable Fisheries Act, it is the responsibility of the National Marine Fisheries Service (NMFS) to safeguard the health of fish in estuarine and coastal waters.
2. This includes assessment of the impacts of exposure to toxic chemicals on fish and their critical habitat.
3. This analysis was conducted to assist NMFS resource managers in determining when fish are exposed to potentially harmful concentrations of one of the most common environmental contaminants, polycyclic aromatic hydrocarbons (PAHs).
4. Effects thresholds were estimated primarily through segmented regression of site-specific sediment PAH concentrations and associated disease prevalences in a resident fish species, English sole, *Pleuronectes vetulus*.
5. The analyses and supporting data encompass several endpoints, including DNA damage, liver lesions, and impacts on growth and reproduction.
6. In general, liver lesion prevalences, DNA adduct levels, and impacts on growth and reproduction were minimal at sediment PAH concentrations at or below 1000 ppb. Above 1000 ppb, there appears to be a substantial increase in the risk of contaminant-related injury to English sole.

KEY WORDS: polycyclic aromatic hydrocarbons; English sole; sediment quality criteria

INTRODUCTION

Under the US Endangered Species Act (ESA) and the Essential Fish Habitat provisions of the Sustainable Fisheries Act (SFA) it is the responsibility of the National Marine Fisheries Service (NMFS) to safeguard the health of fish in estuarine and coastal waters. This includes assessment of the impacts of exposure to toxic chemicals on fish and their critical habitat. Under the ESA, the responsibilities are very specific:

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endangered fish must be protected against any activities that may kill or injure them, or interfere with breeding, spawning, rearing, migrating, feeding, or sheltering (NOAA, 2000). Similarly, under the SFA, the NMFS is charged with managing threats to essential fish habitat, defined as 'those waters and substrate necessary to fish for spawning, breeding, feeding, or growth to maturity' (USDOC, 1996).

Although sediment and water quality standards for both marine and fresh waters are in place in many parts of the United States, these standards are generally designed to protect aquatic life in general, and are based on a limited number of model taxa (USEPA, 1986a). As such, they may not be adequately protective of listed species, or of specific commercially important marine fish covered under the Essential Fish Habitat provisions of the SFA. Moreover, they are often based on laboratory bioassays where mortality is the main endpoint. As a result, they may not be protective against sublethal effects on individuals, which are important under the ESA.

Since the mid-1990s, over 20 United States Pacific Coast salmon and steelhead trout stocks have been listed as threatened or endangered by the NMFS (NMFS, 2000), and the American Fisheries Society has identified 62 marine fish stocks that it considers vulnerable to extinction (Musick *et al.*, 2000). Consequently, the degree to which current water and sediment quality regulations are protective of fish species that are potentially at risk has become a major concern for the NMFS and other agencies that act as trustees for marine and estuarine resources. Our ESA responsibilities, in particular, require us to carefully examine existing practices and regulations, in order to answer questions such as: What are acceptable levels of contaminants in marine and estuarine sediments for minimal biological and ecological damage? What levels are safe for protection of threatened or endangered species? What are realistic target levels for sediment contaminants in remediation projects?

The analysis described in this paper was conducted to assist resource managers in the NMFS in determining when fish are exposed to potentially harmful concentrations of one of the most common environmental contaminants, polycyclic aromatic hydrocarbons (PAHs). PAHs are frequently detected in sediments of Puget Sound and other industrialized embayments worldwide. The majority of PAHs associated with sediments in Puget Sound as well as at other coastal urban sites originate from petroleum and combustion products (Varanasi *et al.*, 1992; MacDonald and Crecelius, 1994). Sources include industrial discharges, creosote from treated wood, municipal run-off, and atmospheric emissions from incineration and automobile emissions. PAHs are also introduced into marine systems through accidental spills of fuel oil, crude oil, and other petroleum products, and from non-point sources.

PAHs, particularly the higher molecular weight compounds, tend to adsorb to organic or inorganic matter in sediments, where they can be trapped in long-term reservoirs. Although only a portion of sediment-adsorbed PAHs are readily bioavailable to marine organisms, there is substantial uptake of these compounds by resident benthic fish through the diet, through exposure to contaminated water in the benthic boundary layer, and through direct contact with sediment. Benthic invertebrate prey are a particularly important source of PAH exposure for marine fishes, as PAHs are bioaccumulated in many invertebrate species (Varanasi *et al.*, 1989, 1992; Meador *et al.*, 1995).

A notable feature of PAHs is that they are metabolized extensively in vertebrates, including fishes, unlike many chlorinated hydrocarbons, which bioaccumulate in tissues. Cellular metabolism of PAHs (Varanasi *et al.*, 1989) results in conversion of these hydrophobic compounds into polar, water-soluble forms that can be readily excreted from the organism. Consequently, although PAHs and their metabolites in invertebrate prey are passed on to consuming fish species (James *et al.*, 1991, McElroy *et al.*, 1991), and PAH–DNA adducts accumulate in the liver of fish chronically exposed to sediment-associated PAHs (Reichert *et al.*, 1998), parent PAHs generally do not bioaccumulate in fish or other vertebrates.

In spite of the fact that they do not bioaccumulate in tissues, PAHs are capable of causing a variety of deleterious effects in exposed animals. While metabolism serves mainly as a pathway of detoxication for PAHs, some of the metabolites that are intermediates in this process possess carcinogenic, mutagenic and

cytotoxic activity. Laboratory tests with rodents, as well as epidemiological studies, have established that certain PAHs, including benzo[*a*]pyrene, benz[*a*]anthracene, dibenz[*a,h*]anthracene, benzofluoranthene, and ideno(1,2,3-*c,d*)pyrene, are chemical carcinogens (NTP, 1999). Some PAHs are also known to be immunotoxic and to have adverse effects on reproduction and development (Klaasen, 1997). Over the past 20 years, a number of studies have been conducted on effects of PAHs on marine fish and other marine biota. These studies show that PAHs exhibit many of the same toxic effects in fish as they do in mammals. For example, liver cancer and related lesions have been documented in several species of wild fish as a result of environmental exposure to PAHs (Moore and Myers, 1994).

Sediment quality standards for PAHs have been established in the State of Washington (WAC, 1995), and national standards are under development by the EPA (USEPA, 2000). However, current sediment quality assessments rely heavily on methods based on bioaccumulation of contaminants in target organisms, such as determination of biota-sediment accumulation factors (BSAFs) or critical body residues (see Meador *et al*., 2002a,b). Consequently, PAH toxicity estimates are frequently based on bioassays with benthic invertebrates, which bioaccumulate PAHs. While these methods are relevant for protection of the prey base for marine and estuarine fish, they may not be suitable for assessing the direct impacts of sediment PAHs on fish because fish metabolize PAHs, and may differ from invertebrates in their sensitivity to PAHs. This analysis attempts to relate sediment PAH concentrations to sublethal biological effects in resident populations of a representative marine benthic fish, English sole (*Pleuronectes vetulus*). The goal is to arrive at a realistic estimate of threshold sediment PAH concentrations that are associated with reductions in fish health.

Of the fish in Puget Sound, English sole is one of the most extensively studied species in pollution monitoring programmes. Several features make it suitable as a sentinel species for studies of the effects of environmental pollution. It is widely distributed along the Pacific coast of the USA, in both urban and non-urban environments. Because of its benthic life history as a juvenile and as an adult, it is particularly likely to take up sediment-associated contaminants. In addition, with the exception of a winter spawning migration, English sole within Puget Sound are relatively sedentary, and show high fidelity to the sites where they are resident (Day, 1976). Consequently, biological effects observed in English sole are generally an accurate reflection of PAH exposure at the sites where they are collected. A large body of data has been generated about the effects of PAHs on this species, in both field and laboratory studies. These data show that English sole from PAH-contaminated embayments are highly susceptible to the development of liver cancer and related lesions (Myers *et al*., 1994, 1998a), and also appear to be prone to a number of other adverse health effects, including reproductive abnormalities, immune dysfunction, and alterations in growth and development (Arkoosh *et al*., 1996; Johnson *et al*., 1998a).

Liver disease, including cancer, is one of the best-documented effects of PAH contamination on English sole in Puget Sound (Myers *et al*., 1987, 1990, 1994, 1998a,b; O'Neill *et al*., 1998; PSWQAT, 2000) and other embayments along the west coast (Myers *et al*., 1994, 1998a). In general, the prevalence of liver disease increases with increasing urbanization (Myers *et al*., 1998a; O'Neill *et al*., 1998). Typically, between 25% and 40% of adult English sole sampled from urban embayments, such as Elliott Bay and Commencement Bay, exhibit neoplastic, preneoplastic, or unique degenerative liver lesions, as compared with 3–8% of adult sole from non-urban and moderately urbanized sites. In several independent statistical analyses of field data from different studies (Rhodes *et al*., 1987, Landahl *et al*., 1990, Myers *et al*., 1990, 1994, 1998a,b), exposure to PAHs was identified as the major risk factor for neoplasms and related liver lesions in English sole. A cause-and-effect relationship between PAHs and toxicopathic liver lesions in English sole is supported by induction of degenerative, proliferative, and preneoplastic lesions, identical to those observed in field-collected fish, when sole are exposed in the laboratory to model carcinogenic PAHs such as benzo[a]pyrene (BaP) and to extracts of sediments from PAH contaminated sites (e.g. Eagle Harbor) in Puget Sound (Schiewe *et al.,* 1991) where lesions are observed.

In addition to toxicopathic liver disease, English sole residing in contaminated areas in Puget Sound also suffer from various types of reproductive impairment. Field studies show that female English sole from areas with high concentrations of PAHs in sediment are less likely to enter vitellogenesis and have lower plasma concentrations of the female reproductive hormone, 17β-estradiol, than sole with low levels of contaminant exposure (Johnson *et al.*, 1988, 1997, 1999). At minimally contaminated sites within Puget Sound (sediment total PAH concentrations < 500 ppb dry wt), approximately 80–90% of adult females undergo gonadal development (Johnson *et al.*, 1988, 1991, 1999; Sol *et al.*, 1999), while at more highly contaminated sites (e.g., the Duwamish and Hylebos Waterways and Eagle Harbor) the percentage declines to 40–60%. In statistical analyses of these data, exposure to PAHs emerges as a major risk factor for inhibited ovarian development in adult sole. English sole from PAH-contaminated areas also display increased ovarian atresia and reduced egg production (Johnson *et al.*, 1988, 1997). Results of these field studies are supported by laboratory experiments showing that pretreatment of gravid female English sole with extracts of contaminated sediment or crude oil containing high levels of PAHs decreased levels of endogenous estradiol (Stein *et al.*, 1991; Johnson *et al.*, 1995). More recent experiments suggest that exposure to benzo[*a*]pyrene or PAH-contaminated sediment may suppress estradiol-induced vitellogenin production in English sole (Anulacion *et al.*, 1997).

Studies also suggest that English sole from PAH-contaminated areas that do successfully enter vitellogenesis may experience inhibited spawning ability and reduced egg viability. When gravid English sole from four Puget Sound sites (Port Susan, Sinclair Inlet, the Duwamish Waterway, and Eagle Harbor) were brought into the laboratory and artificially induced to spawn, spawning success was significantly lower in fish from the two most contaminated sites, Eagle Harbor and the Duwamish Waterway (Casillas *et al.*, 1991). Moreover, exposure to PAHs in the water column (e.g., fluoranthene at 0.075–7.5 mg of PAH lit^{-1} of seawater) caused larvae to become disoriented and to exhibit signs of narcosis, with mortality at the higher concentrations (Eddy *et al.*, 1993).

Exposure to PCBs may also contribute to reproductive problems in female English sole, as PCBs often co-occur with PAHs at contaminated sites, and are known reproductive toxicants (Brouwer *et al.*, 1995). In several studies (Johnson *et al.*, 1997, 1999; Casillas *et al.*, 1991), exposure to PCBs and other organ ochlorine compounds was highly correlated with reduced larval viability, reduced egg size, and precocious sexual maturation in female English sole. However, PAH exposure was more highly correlated than PCB exposure with inhibited gonadal development, inhibited spawning, and reduced egg quality: reproductive problems especially prevalent in sole from Eagle Harbor, a site with high concentrations of PAHs, but minimal PCB contamination (Johnson *et al.*, 1988; Casillas *et al.*, 1991).

Effects of PAHs on reproduction have not been studied as extensively in male as in female English sole. There is evidence, however, that males may also be susceptible to PAH-related reproductive dysfunction. Preliminary studies suggest that although testicular development in male sole from PAH-contaminated sites is relatively normal, plasma concentrations of 11-ketotestosterone and testosterone are reduced in fish with particularly high concentrations of PAH metabolites in bile, a measure of PAH exposure (Sol *et al.*, 1999). In other fish species, PAH exposure has been associated with reduced sperm production and reduced sperm quality (Nagler and Cyr, 1997), but these parameters have not been measured in English sole.

English sole growth also appears to be affected by exposure to PAHs. Recent laboratory studies (Kubin, 1997; Rice *et al.*, 2000) show reduced growth in juvenile English sole exposed to PAHs through contaminated sediment or diet. These effects have not yet been corroborated in wild populations of English sole, but if they do occur, they could impact sole populations by reducing fecundity or altering the time to sexual maturity (Brandt *et al.*, 1992). Slow growth rates have also been associated with increased juvenile mortality in several fish species (Peterson and Wroblewski, 1984; Lorenzen, 1996; McGurk, 1996).

ANALYSIS

The purpose of the following analysis was to use existing data on PAH effects in English sole to determine sediment PAH concentrations at which biological injury is likely to occur. We selected English sole as the target species because of its sensitivity to PAHs, its presence at sites within Puget Sound where resource damage assessment and restoration efforts are planned or underway, and because there is substantial information on health effects of PAHs in this species from field studies, which have been corroborated, in large part, by laboratory studies. Our initial focus was on liver lesions in English sole, because of the abundance of data available for this health effect, and the preponderance of evidence for a cause and effect relationship between PAHs and the development of liver cancer in English sole. However, we also include data on DNA damage, reproductive effects and impaired growth.

For this analysis, biological effects were linked directly to sediment PAH concentrations at sites where test animals were resident, rather than to contaminant body burdens. This was done primarily because PAHs are metabolized in fish and do not bioaccumulate (Varanasi *et al.,* 1989), but also because the large volume of field data available on site-specific sediment PAH concentrations and associated biological effects in English sole made such an analysis feasible. Additionally, the sediment-based analysis is more directly applicable to the development of appropriate sediment quality guidelines for the protection of estuarine fish. An advantage of using field data for the analyses is that threshold estimates are based on health effects measured in a native organism collected from its natural environment. As such, there are fewer questions as to the environmental relevance of this approach, compared to laboratory bioassays. The effects considered reflect long-term exposure, potentially over the life of the organism, and incorporate exposure through all routes of uptake, including diet, water, and directly from sediment through skin contact or ingestion. In laboratory exposure studies, exposure generally lasts for only a limited period of time, and uptake is typically restricted to a single route of exposure, such as diet, or direct contact with sediments. A disadvantage of the field-based approach is that it may not adequately account for biological effects resulting from exposure to other contaminants or contaminant mixtures present in sediments. However, this can be mitigated to some extent by choosing endpoints, such as liver cancer and related lesions, for which PAHs are known to be a strong causative factor.

Liver disease

National Benthic Surveillance Project (NBSP) data set: Using data collected over the past 10 years from a variety of field studies in Puget Sound and on the West Coast (Myers *et al*., 1998a; Brown *et al*., 1998; McCain *et al*., 2000), we statistically determined threshold PAH concentrations at which liver lesion prevalences begin to increase. The hockey stick regression model (Horness *et al*., 1998) was employed in these analyses. Hockey stick regression is one of a number of standard dose-response models (Gad and Weil, 1991), and has been used in a variety of epidemiological and toxicological studies (e.g. Hammer *et al*., 1974; Cox *et al*., 1989; Gordon and Fogelson, 1993). The model consists of two linear segments whose 'blade and handle' shape resembles a hockey stick (Yanagimoto and Yamamoto, 1979). In the present application (Figure 1), the lower segment was assigned a slope of zero to represent a constant low-level background effect. The upper segment was defined as a linear function with a positive slope. The upper segment represents a dose–response relationship above a threshold that is estimated by the point of intersection of the two segments, as follows:

$$\text{Effect} = \text{background for SC} < \text{SC}_\text{T}$$

$$\text{Effect} = \text{background} + \beta(\text{SC} - \text{SC}_\text{T}) \quad \text{for SC} > \text{SC}_\text{T}$$

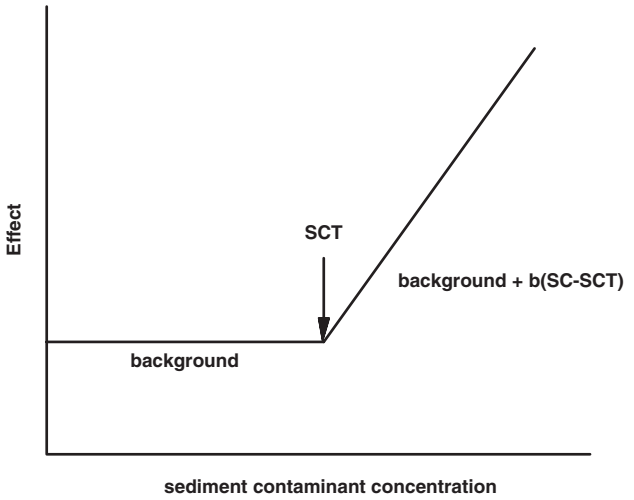


Figure 1. Generalized hockey-stick regression model relating biological effect to sediment contaminant concentration. SC = sediment contaminant concentration, SCT = threshold sediment contaminant concentration at which the biological effect begins to increase above background level.

where SC is the sediment contaminant concentration, SC_T the threshold sediment contaminant concentration.[1]

SC_T (threshold contaminant concentration), background (the spontaneous background effect) and β (the slope of the curve) are the estimated parameters. Effect and SC (sediment contaminant concentration) are the dependent and independent variables, respectively.

We realize that the risk analysis models used in epidemiology typically assume that cancer induction is a non-threshold phenomenon, and our choice of a threshold model for this exercise is not meant to imply that a true threshold exists in the process of carcinogenesis in English sole. Rather, the model was chosen for pragmatic reasons, to facilitate our identification of exposure levels at which statistically detectable and biologically relevant increases in lesion prevalence would be expected to occur in wild fish populations. The application of a threshold model is supported by the fact that, for most carcinogens, repair processes and compensatory mechanisms exist that can counteract the effects of carcinogens at low levels of exposure, even though one molecule of a carcinogen could theoretically induce an initiated cell, leaving no latitude for a threshold. Based on a similar rationale, the application of a threshold approach for regulating exposure to some carcinogens has been suggested for human health risk management (Lutz, 1998; Butterworth and Bogdanffy, 1999; Gaylor *et al*., 1999).

The model was used to relate sediment PAH concentrations to prevalences of the four most common toxicopathic hepatic lesion types found in English sole. These four types were (1) neoplasms; (2) preneoplastic foci of cellular alteration (FCA), which are thought to be precursors of neoplasms; (3) specific degeneration/necrosis (SDN), a degenerative lesion associated with exposure to PAHs; and (4) non-neoplastic proliferative lesions, such as hyperlasia of hepatocytes or bile ducts. Additionally, a composite category was examined consisting of the presence of one or more of any of the first three lesions (neoplasms, FCA, and SDN) in an individual fish. Lesion prevalences were based on examination of between 30 and 60 fish per site. Collection site locations are described in detail in Horness *et al*. (1998) and

[1] The notation SC_T (threshold sediment contaminant concentration) is used in this paper to be consistent with Horness *et al*. (1998), but in application this is equivalent to SET (sediment effect concentration) used in Meador *et al*. (2002a,b) which are in this volume.

Myers *et al*. (1994). Because age is a significant risk factor for preneoplastic and neoplastic lesions in English sole, all fish less than 2 years of age were excluded from prevalence calculations. This exclusion is based on data indicating that fish below this age have not had sufficient time to develop these types of hepatic lesions even at high exposure levels (Myers *et al*., 1998b).

To characterize the level of contamination present at a given site, surficial sediment samples were collected from the area in which the fish were captured and analysed for toxic contaminants (Sloan *et al*., 1993) including both low and high molecular weight PAHs (Table 1). The correlation between the low and high molecular weight analytes was too high to consider them as separate factors ($r^2 = 0.94$). Moreover, there is only limited direct information to link a specific subset of PAHs to the effects that have been observed. Consequently, total sediment PAH content (the sum of all analytes shown in Table 1) was used as the contaminant category of interest for this analysis. It should be noted that the sediment samples used in this study contained not only lower molecular weight compounds associated with oil and other petroleum products, but significant concentrations of higher molecular weight PAHs which are typical of industrial urban sites (Brown *et al*., 1998). Consequently, sediment PAH effects thresholds generated with these data may not be fully applicable at sites where sediments contain primarily low molecular weight PAHs, whose carcinogenic potency is generally not as great as that of high molecular weight PAHs (NTP, 1999).

As contaminant concentration data are generally log-normally distributed, the PAH concentrations were log-transformed prior to the regression. Thus, log (sediment PAH concentration) was used for the independent variable SC. Lesion prevalence was used for the dependent variable (Effect). The model was fitted using a non-linear regression parameter estimation routine from the SAS statistical package JMP. Confidence intervals were computed at an α of 0.1, rather than the standard 0.05, to increase the statistical power to detect a threshold and decrease the likelihood of a type II error (false negatives) without greatly increasing the likelihood of type I errors (false positives) (Peterman, 1990).

Table 1. Polycyclic aromatic hydrocarbons (PAHs) measured in sediment samples included in determination of PAH effect thresholds for liver lesions in English sole for NBSP studies (Brown *et al*., 1998; McCain *et al*., 2000) and PSAMP studies (PSWQAT, 2000; Myers and O'Neill, in preparation)

NBSP List	EPA List
Low molecular weight (2–3 rings)	
Naphthalene	naphthalene
2-Methylnaphthalene	2-Methylnaphthalene
Acenaphthene	acenaphthene
Fluorene	fluorene
Phenanthrene	phenanthrene
Anthracene	anthracene
Biphenyl	acenaphthalene
1-Methylnaphthalene	
2,6-Dimethylnaphthalene	
1-Methylphenanthrene	
High molecular weight (4–6 rings)	
Fluoranthene	Fluoranthene
Pyrene	Pyrene
Benz[*a*]anthracene	Benz[*a*]anthracene
Chrysene	Chrysene
Benzo[*a*]pyrene	Benzo[*a*]pyrene
Perylene	Benzo(*g*,*h*)perylene
Dibenzo[*a*,*h*]anthracene	Dibenzo(*a*,*h*)anthracene
Benzo[*e*]pyrene	Benzofluoranthene
	Ideno(1,2,3-*c*,*d*)pyrene

Depending on the specific type of lesion, threshold effect estimates ranged from 54 to 2800 ppb (ng g^{-1} dry wt.) (Figures 2(a–d); see Table 2 for regression parameters). All threshold estimates were statistically significant ($\alpha = 0.1$) except for that for FCA for which no lower confidence bound was found. At 2800 ppb (90% CI = 11–5500 ppb), neoplasms exhibited the highest PAH threshold. FCA exhibited the lowest threshold (54 ppb; CI = 0–870 ppb) and the lack of a lower confidence limit suggests that FCA prevalence in English sole may be directly proportional to sediment PAH content over the entire range of contaminant concentration rather than operating through a threshold relationship. The calculated threshold concentration for SDN, the most common toxicopathic lesion in English sole, was 940 ppb (CI = 600–1400 ppb).

DNA damage

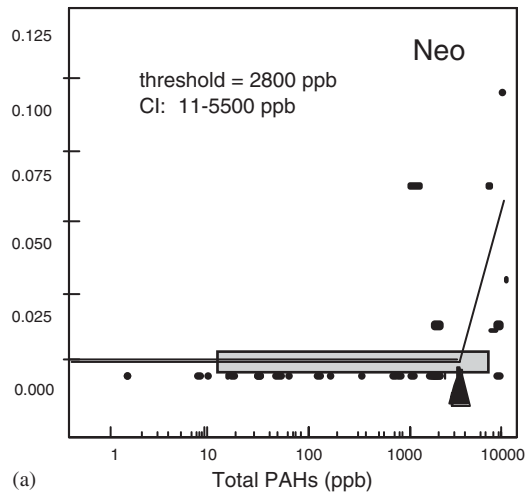
The threshold analysis for DNA damage was done in the same manner as the analyses for liver lesions, but using the mean concentration of aromatic compound–DNA adducts in liver of English sole from the sampling sites as the outcome variable (i.e. Effect). As with liver lesions, our application of a threshold model for this analysis does not imply that DNA adduct formation itself is a threshold phenomenon. Our objective was to use the model to assist us in identifying the PAH exposure level where increases in mean DNA adduct levels in liver could first be detected in English sole populations. Mean DNA adduct levels were based on analyses of liver tissue from 3 to 10 fish per site. The threshold effect estimate was 290 ppb (ng/g dry wt.), with a 90% CI of 6–1380 ppb (Figure 3; see Table 2 for regression parameters). This threshold estimate is at the lower end of the range of threshold estimates for liver lesions, which is toxicologically consistent with the hypothesis that this type of DNA damage is a precursor to more overt pathological conditions in the liver, and a necessary step in the development of certain types of neoplasms (Farber and Sarma, 1987). A threshold in this range is also supported by a laboratory study (French *et al.*, 1996) in which exposure to sediments contaminated with 1200 ng g^{-1} dry wt. PAH resulted in DNA adduct concentrations in English sole liver of 15–20 adducts mol^{-1} nucleotides, in comparison with 5–6 adducts mol^{-1} nucleotides in fish exposed to sediments containing 20 ng g^{-1} PAH. The threshold model predicts adduct levels of 28 adducts/mol nucleotides at 1200 ng g^{-1} dry wt. PAH, and 5 adducts/mol nucleotides and 5 ng g^{-1} dry wt. PAH. The laboratory study, however, did not include any exposure treatments between 20 ng/g and 1200 ng g^{-1} PAH. Additional data on DNA adduct levels in sole exposed to sediments with PAH concentrations in the 100–1000 ppb range would be necessary to provide laboratory validation of the threshold estimate.

DNA adducts represent a tissue-level alteration or injury that is caused by PAH exposure, is correlated with other health effects such as liver disease, and can develop with relatively short-term exposure of days to weeks (Reichert *et al.,* 1998). In addition, hepatic DNA adducts in fish are persistent and accumulate with chronic exposure to sediment-associated PAHs (French *et al.,* 1996; Reichert *et al.*, 1998). As such, DNA adducts can be used as an indicator of PAH exposure and effects for estimating the likelihood of PAH-induced injury in species that are short-term residents of urban estuaries, such as anadromous salmonids.

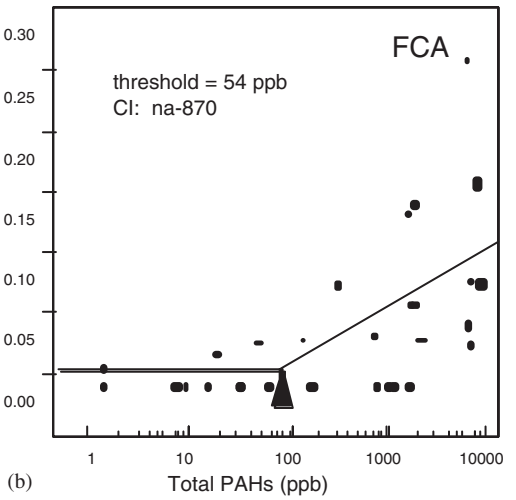
Reproductive dysfunction

Although we do not have sufficient data to statistically determine precise thresholds for other effects in English sole, available information indicates that additional types of impairment begin to occur at sediment

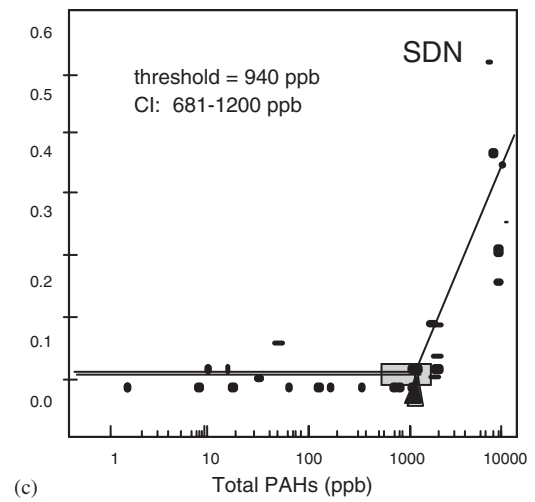
Figure 2. Hockey-stick regressions of hepatic lesion prevalence in English sole versus total polycyclic aromatic hydrocarbons (PAH) in bottom sediment in ng/g dry wt. (ppb) for (a) neoplasms (Neo); (b) foci of cellular alteration (FCA); (c) specific degenerative/necrosis (SDN); lesions; (d) proliferative lesions (Prolif); (e) and either Neo, FCA, or SDN (any lesion). Arrows indicate threshold concentrations. Shaded grey bar represents the 90% confidence interval. No lower confidence found was found for the FCA threshold estimate. $n = 29$. Data from Horness *et al.* (1998).


Neo
threshold = 2800 ppb
CI: 11-5500 ppb
0.125
0.100
0.075
0.050
0.025
0.000
1
10
100
1000
10000
Total PAHs (ppb)

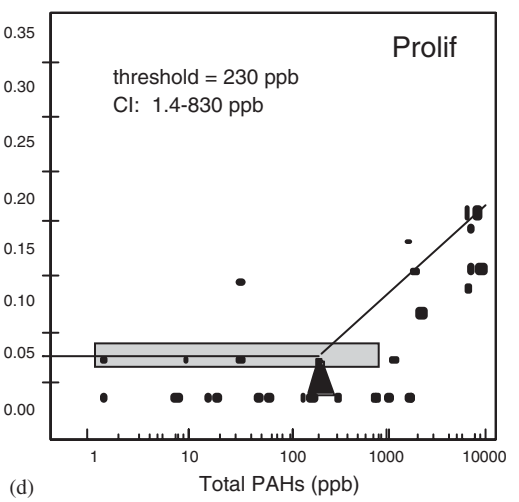
(a)


FCA
threshold = 54 ppb
CI: na-870
0.30
0.25
0.20
0.15
0.10
0.05
0.00
1
10
100
1000
10000
Total PAHs (ppb)

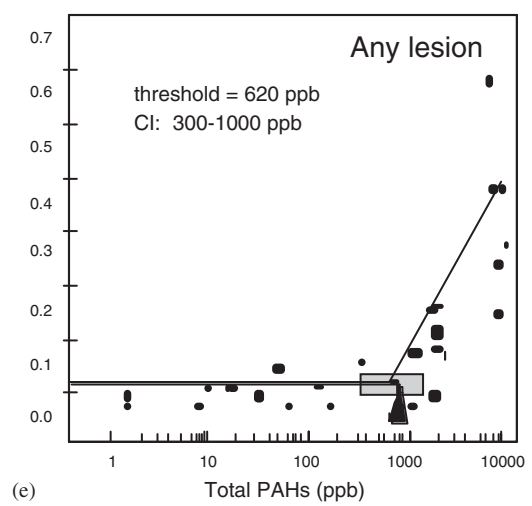
(b)


SDN
threshold = 940 ppb
CI: 681-1200 ppb
0.6
0.5
0.4
0.3
0.2
0.1
0.0
1
10
100
1000
10000
Total PAHs (ppb)

(c)


Prolif
threshold = 230 ppb
CI: 1.4-830 ppb
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
1
10
100
1000
10000
Total PAHs (ppb)

(d)


Any lesion
threshold = 620 ppb
CI: 300-1000 ppb
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
1
10
100
1000
10000
Total PAHs (ppb)

(e)

Table 2. Parameter estimates for hockey stick regression of the relationship of sediment polycyclic aromatic hydrocarbon (PAH) concentration (dry weight) with hepatic lesions and reproductive abnormalities in English sole. Adapted from Horness *et al.* (1998).

Effect[a]	Hockey stick regression parameter estimates				Back-transformed threshold estimates	
	Threshold[b] (log ppb)[c]	Threshold confidence limits (log ppb)[c]	Background[d]	Rate of increase (β)[e]	Threshold (ppb)[c]	Threshold confidence limits (ppb)[c]
DNA damage	2.46	0.75–3.09	4.5	38	288	6–1318
Liver lesions						
Neo	3.45	1.04–3.75	0.004	0.10	2800	11–5500
FCA	1.74 (ns)[f]	na[g]–2.94 (ns)[f]	0.008	0.04	54 (ns)[f]	na[g]–870
SDN	2.97	2.75–3.16	0.013	0.37	940	600–1400
Prolif	2.37	0.14–2.92	0.024	0.09	230	1.4–830
Any lesion	2.79	2.48–3.01	0.024	0.31	620	300–1000
Reproductive abnormalities						
Inhibited gonadal growth	3.6	nd[h]	0.15	0.31	4000	nd[h]
Inhibited spawning	2.8	nd[h]	0.12	0.26	630	nd[h]
Infertile eggs	2.8	nd[h]	0.38	0.19	630	nd[h]
Abnormal larvae	2.8 (ns)[f]	nd[h]	0.25	0.12 (ns)[f]	630 (ns)[f]	nd[h]

[a] For all liver lesions, inhibited gonadal growth, and inhibited spawning, effect endpoints are sampling site prevalences. For DNA damage, endpoint is mean concentration of PAH–DNA adducts in liver of fish from the sampling site. For infertile eggs and abnormal larvae, endpoints are the average proportion of spawned eggs that were infertile or proportion of larvae that were abnormal in females from Puget Sound sampling crossed with reference males. Neo = neoplasms, FCA = foci of cellular alteration, SDN = specific degeneration/necrosis, Prolif = proliferative lesions, any lesion = Neo or FCA or SDN, immature = failing to undergo gonadal development, Non-spawning = failing to spawn; Infertile eggs = proportion of spawned eggs that not fertilized, Abnormal larvae = proportion of hatched larvae with developmental abnormalities.

[b] For liver lesions, thresholds are estimated from hockey-stick regression; for reproductive abnormalities, threshold = the geometric mean of the highest sediment PAH concentrations where the effect level was at background and the lowest sediment PAH concentration where an increase in effect level was seen.

[c] Measurement units in ppb = sediment PAH concentration in ng/g dry wt. sediment.

[d] For all liver lesion categories, Immature and Non-spawning, units for background values are prevalence in frequency of occurrence; for infertile eggs and abnormal larvae, units are proportion of eggs or larvae affected. For DNA adducts, units are number of adducts per nmol of bases. For liver lesions and DNA adducts, background was estimated using hockey-stick regression; for reproductive abnormalities, background = the effect prevalence or proportion at the reference sites with the lowest sediment PAH concentration (see Johnson *et al.*, 1988, 1991, 1999; Casillas *et al.*, 1991; Sol *et al.*, 1998).

[e] Rate of increase computed as the increase per unit increase in log (PAH concentration in ppb). For reproductive abnormalities, threshold and background effect level estimates from field data were substituted into the hockey-stick regression model for computation of β.

[f] ns = not significant; the confidence interval does not lie entirely within the data range, or for β, does not exclude 0. The threshold estimate for Abnormal larvae was not calculated through regression, but is considered non-significant because of the lack of a significant positive correlation between sediment PAH concentration and proportion of abnormal larvae.

[g] na = not available; the confidence interval is unbounded in this direction.

[h] nd = not determined; sufficient data were not available for calculation of this parameter.

PAH concentrations similar to those associated with liver lesion occurrence and DNA damage. For example, in Figure 4, prevalences of several types of reproductive impairment observed in female English sole (Johnson *et al.*, 1988, 1999; Casillas *et al.*, 1991) are plotted against sediment PAH concentrations at the sites where fish were collected in Puget Sound. All fishes utilized in these analyses were of reproductive size and age, at least 3 years of age or 30 cm in length, which is when female English sole typically reach maturity (Garrison and Miller, 1982; Johnson *et al.*, 1991). The plots indicate that spawning failure and egg infertility begin to increase above background levels at sediment PAH concentrations between 250 and 1600 ppb, while the proportion of sole failing to undergo ovarian maturation begins to increase at

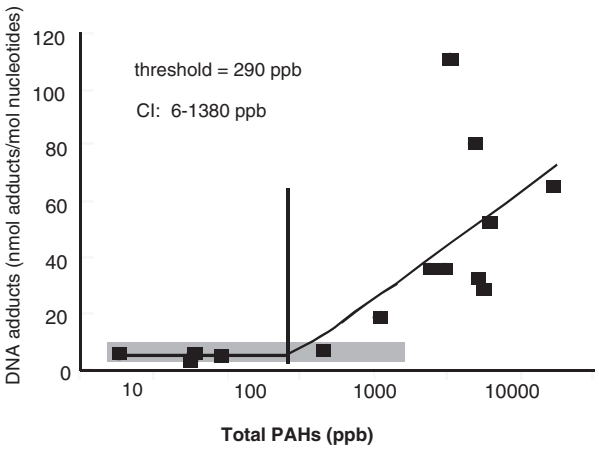


Figure 3. Hockey-stick regression of PAH–DNA adducts (nmol adducts per mol nucleotides) in liver of English sole versus total polycyclic aromatic hydrocarbons (PAHs) in bottom sediments in ng/g dry wt. (ppb) for selected sampling sites in Puget Sound, Washington. Threshold concentration is indicated by vertical line. Shaded gray bar represents the 90% confidence interval. Data from Stein *et al*. (1992); French *et al*. (1996); Collier *et al*. (1998); French (unpublished data).

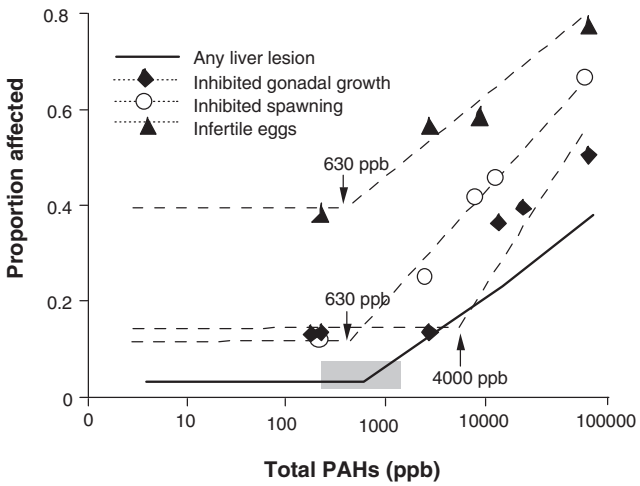


Figure 4. Measures of reproductive function in female English sole plotted against sediment total PAH concentrations at sites in Puget Sound where sole were collected. For inhibited gonadal growth and inhibited spawning, data points represent the proportion of females at the sampling site that exhibited these conditions. For infertile eggs, the data points represent the average proportion of spawned eggs that were infertile in crosses with sperm from reference males for females from the sampling sites. Data are from Johnson *et al*. (1988, 1999); Casillas *et al*. (1991); Collier *et al*. (1998). The dotted lines indicate the hypothetical hockey-stick regression models for reproductive endpoints. The horizontal portion of the curve represents background values for these endpoints, estimated from field data at the reference site with the lowest sediment PAH concentration. Threshold concentrations (indicated by arrows) are the geometric mean of the highest sediment PAH concentration where effect values were at the background level, and the lowest concentration where an increased effect level was observed. The upper portion of the curve is the fitted hockey-stick regression model. Estimated threshold and background effect levels were substituted into the model, rather than calculated through regression analysis as for liver lesions. The solid black line represents the fitted hockey-stick regression model and effects threshold estimate for any lesion in English sole (neoplasms, foci of cellular alteration, or specific degenerative necrosis; Horness *et al*., 1998).

concentrations between 1600 and 10 000 ppb. If we estimate the effect thresholds by taking the geometric mean of these two points, this yields an effect threshold of 4000 ppb for inhibition of ovarian development, and a threshold of 630 ppb for all other reproductive endpoints.

Exposure–response relationships for reproductive endpoints at sediment PAH concentrations above the threshold levels were estimated using the formula for fitting the upper (sloped) segment of the hockey-stick regression:

$$\text{Effect} = \text{background} + \beta(\text{SC} - \text{SC}_\text{T})$$

Estimated threshold and background effect levels were substituted into the model, rather than calculated through regression analysis as for liver lesions and DNA adducts. Background effect levels were assumed to be identical to those found at reference sites with the lowest sediment PAH concentrations (see Johnson *et al*., 1988, 1991, 1999; Casillas *et al*., 1991; Sol *et al*., 1999), so that background = 0.15 for proportion failing to mature; 0.12 for proportion failing to spawn; 0.25 for proportion of abnormal larvae; and 0.38 for proportion of infertile eggs. The estimated SC_T's used for this analysis were 4000 ppb for failing to mature and 630 ppb for all other endpoints, as described above. Parameter estimates derived from the regression analysis are shown in Table 2, and plotted regression curves are shown in Figure 4. The horizontal portion of the curve represents background values for these endpoints, estimated from field data, while the upper portion is the fitted hockey stick regression model.

The results show that the proportion of sole that failed to mature, the proportion that failed to spawn and the proportion of eggs spawned that were infertile were all significantly correlated with increasing sediment PAH concentration, as indicated by positive estimates for β with confidence intervals that excluded zero. However, the proportion of abnormal larvae produced showed no clear relationship with sediment PAH level; the confidence interval for β included zero, indicating that the slope of the line describing the relationship between sediment PAH concentration and the proportion of abnormal larvae was not statistically different from zero. Consequently, this endpoint was excluded from further analyses of PAH effect thresholds and exposure–response relationships.

While more data would be needed to calculate sediment concentration thresholds and confidence intervals (SC_T's and CI's) for the reproductive endpoints, this analysis currently provides the best available threshold estimates to compare to the SC_T's for liver lesions in English sole. The results also suggest that these types of reproductive impairment are found at sediment PAH concentrations similar to those associated with the development of hepatic lesions.

Growth reduction

Data from two recent laboratory exposure studies suggest that declines in growth of juvenile English sole also occur from exposure to PAHs (Kubin, 1997; Rice *et al*., 2000). Although neither of these experiments were designed specifically to identify effects thresholds or model dose response relationships, the results provide information on PAH exposure levels associated with growth impairment in sole. Kubin (1997) exposed juvenile English sole to sediments contaminated with PAHs at concentrations of approximately 4000, 2000 and <50 ppb dry wt. for 6 months.[2] For the first 3 months, growth rates were similar for all treatments (1.0–1.1% per day for weight, and 0.36–0.38% per day for length). However, during the next 3 months, growth rates were significantly lower in the high exposure group; relative to control fish, a 19% reduction in growth rate, measured by either weight or length, was observed. The fish exposed to sediments with moderate PAH concentrations showed no significant decrease in growth rate relative to control fish. These data suggest a threshold for growth effects in the 2000–4000 ppb range. Actual threshold effect concentrations could be lower, as in this experiment, uptake of PAHs was from sediment and water only,

[2] Sediment PAH concentrations for this study were determined using the HPLC/PDA screening system of Krahn *et al*. (1991), which provides an estimate of PAH concentration by measurement of fluorescent aromatic compounds (FACs) in sediments. Total PAH concentrations, as determined by GC/MS, were estimated based on data from Krahn *et al*. (1988), in which parallel sediment samples from a variety of urban and non-urban sites along the US West Coast were analysed using both methods.

and in the natural environment, substantial exposure would also occur through the diet, with ingestion of invertebrate prey species residing in contaminated sediment.

A study by Rice *et al.* (2000) confirmed both the effect of PAHs on growth of juvenile English sole and the importance of dietary exposure. The findings showed significantly reduced weight in juvenile English sole fed polychaete worms reared on sediments containing 3000–4000 ppb dry wt. of PAHs, after an exposure period of only 12 days. The growth rate was markedly lower (0.05-0.1% per day) in exposed fish than in control fish (1.1–1.2% per day). If sole had been exposed to PAHs through uptake from both sediment and diet, as they are in the natural environment, and for a more extended period, it is reasonable to assume that effects on growth could be observed at lower sediment PAH levels, more comparable to the SCT for liver lesions. If, for example, a safety factor (Mount, 1977) of 2 were applied to the 3000 ppb sediment, PAH level associated with growth reductions in Rice *et al.* (2000), to account for additional PAH uptake through sediment and the water column, this would yield a threshold concentration of 1500 ppb, which is comparable to threshold concentrations associated with liver lesions, DNA damage, and certain reproductive effects.

The central finding from these data is that English sole exposed to PAHs in sediments at concentrations where toxicopathic lesions are observed are also likely to experience negative impacts on growth and reproduction. All three types of effects represent significant injury to the health of affected fish. The impacts on growth and reproduction are particularly likely to affect the productivity of stocks from contaminated sites because of their potential to reduce fecundity and age of sexual maturation.

SOURCES OF UNCERTAINTY IN THRESHOLD ESTIMATES

Although we believe that the present analysis provides good guidance on approximate sediment PAH concentrations associated with injury in English sole, several variables that could influence exposure–response relationships cannot be fully accounted for in this analysis of current data. Among the more important factors are fish age, length of exposure, relative proportions of high and low molecular weight PAHs in sediments, exposure to other contaminants, and species sensitivity, if the analysis is to be used to extrapolate to effects on other species.

The present analysis adjusts for fish age only in a very basic manner. Very young fish were excluded, as well as sites where the majority of fish collected were sub-adults. Because the analysis was done on a site basis rather than on individual fish basis, it was not possible to fully adjust for the increasing risk of disease development with age. Moreover, the number of animals lost to disease is not known, and is not incorporated into the analysis. Because of these limitations, the analysis may underestimate the lifetime cancer risk to English sole of a particular PAH exposure concentration.

The suite of PAHs used to calculate total PAH concentrations for the development of effects thresholds in this analysis differs somewhat from the group of 16 priority PAHs recommended by EPA (USEPA, 1986b; Table 1). Recalculation of the thresholds with this alternate group of PAHs could yield different threshold estimates. However, preliminary analyses of sediment PAH thresholds for liver lesions in English sole have been performed using data from Washington State's Puget Sound Ambient Monitoring Program, in which total PAHs are calculated based on EPA's set of 16 compounds. Liver lesion thresholds, calculated as described in Horness *et al.* (1998), ranged from 500 to 2000 ppb dry wt total PAH in sediment (O'Neill *et al.*, 1998; Myers and O'Neill, in preparation). The threshold for SDN, for example, was 540 ppb dry wt total PAHs (Figure 5). These thresholds are very similar to those derived for English sole using our NBSP data set. These results suggest that the thresholds we have determined are not highly dependent on the exact suite of individual PAHs used to calculate total PAHs, assuming that commonly occurring carcinogenic and mutagenic PAHs are included.

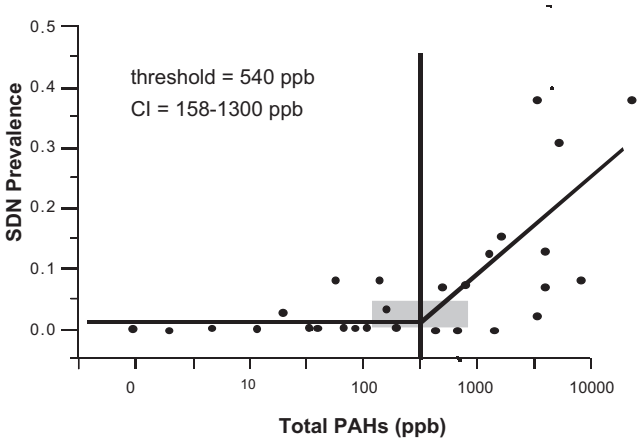


Figure 5. Hockey-stick regressions of hepatic lesion prevalence in English sole versus total polycyclic aromatic hydrocarbons (TPAH) in bottom sediment in ng/g dry wt. (ppb) for specific degeneration/necrosis (SDN), based on data collected as part of the State of Washington's Ambient Monitoring Program. Threshold concentration is indicated by vertical line. Data from O'Neill *et al.* (1998), Myers and O'Neill (in preparation). Total PAHs include the 16 priority PAHs delineated by the United States Environmental Protection Agency (USEPA, 1986; also see Table 1).

A more serious concern is the fact that the applicability of these thresholds to estuarine environments where the suite of PAHs present differs substantially from those typically present at industrialized urban sites (e.g. sites with a preponderance of lower molecular weight petrogenic PAHs) is not known. One way in which this question could be explored would be to express sediment PAH concentrations in terms of BaP equivalents, giving heavier weight to the high molecular weight, carcinogenic and mutagenic PAHs that are thought to be primarily responsible for the development of liver disease in English sole (Myers *et al.*, 1998). However, the extent to which high and low molecular weight PAHs contribute to other biological effects, such as reduced growth and reproductive dysfunction, is less clear, so for these endpoints, such an analysis may be less appropriate. Additional analyses with a variety of data sets would be needed to better establish the applicability of these threshold estimates in other estuarine environments.

The co-occurrence of other contaminants along with PAHs at sites where English sole were collected is not incorporated into the analysis. While the correlations between PAH exposure and the endpoints we measured in this study are statistically valid and well-supported by other scientific evidence, sediments at the sites included in the analysis contain a variety of other compounds (e.g. PCBs, chlorinated pesticides, and heavy metals; see Malins *et al.* (1982); Brown *et al.* (1998); Coller *et al.* (1998); Meador *et al.* (1994) for more detailed information) that are promotors of carcinogenesis, or reproductive toxicants. These compounds could act either additively or synergistically with PAHs to produce the observed health impacts. Their presence could alter disease prevalence, and they are likely an important factor contributing to variability in response among fish populations at different sampling sites. Incorporating the effects of co-occurring chemicals into PAH threshold estimates is beyond the scope of the present analysis. However, the potential of interactive effects among co-occurring compounds is an area that warrants additional research because it does introduce uncertainty in the sediment threshold values.

The present analysis establishes PAH sediment thresholds for protection of a single species, English sole. Their application to other estuarine fish is not known. Species differences in sensitivity to the effects of PAHs are well-documented (Collier *et al.*, 1992, 1993; Johnson *et al.*, 1992, 1998a; Anulacion *et al.*, 1998; Myers *et al.*, 1998a), so variation in PAH effects thresholds would be expected. However, previous research with English sole suggests it is relatively sensitive to PAH-associated injury (Collier *et al.*, 1992; Anulacion

et al., 1998; Johnson *et al*., 1998b; Myers *et al*., 1998a), so sediment thresholds developed for this species may be protective of other fish as well. We have conducted limited preliminary analyses with other bottomfish species, which suggest that sediment PAH thresholds for some categories of non-neoplastic proliferative and degenerative lesions are similar to those for English sole (Lomax *et al*., 1994). For example, sediment PAH threshold concentrations associated with the development of hydropic vacuolation were determined for winter flounder collected as part of the NBSP on the east coast of the United States (Johnson *et al*., 1992, 1994). Hydropic vacuolation is a toxicopathic liver lesion that does not occur in English sole, but is commonly associated with PAH exposure in other species, including winter flounder, starry flounder, white croaker, and rock sole (Moore and Myers, 1994; Stehr *et al*., 1998; Myers *et al*., 1998a). The threshold for this lesion, estimated according to Horness *et al*. (1998), was 298 ppb total PAH (Figure 6), well within the range of values observed for liver lesions in English sole. While these results suggest that a variety of bottomfish species may exhibit adverse health effects at PAH concentrations similar to those that affect English sole, a more thorough investigation of species differences in PAH sensitivity is needed.

The applicability of the standards developed for English sole to pelagic species, including anadromous salmonids, is more difficult to assess. Because these species often spend less time in contaminated estuarine sites, have less contact with sediments, and do not rely as heavily on benthic invertebrates as prey items, their exposure may be considerably less in comparison to bottomfish at sites of comparable sediment PAH concentration. Levels of DNA adducts in liver of juvenile salmonids collected from the Hylebos Waterway, for example, were approximately 10 nmol per mol DNA bases, while adduct levels in English sole from this waterway ranged from 30 to over 100 nmol per mol DNA bases (Collier *et al*., 1998). As yet we do not have sufficient data to estimate threshold sediment PAH concentrations associated with reduced growth or suppressed immune function in juvenile salmonids. However, we can say that these effects have been observed in fish collected from sites with sediment total PAH levels in the 5000–10000 ppb range (Arkoosh *et al*., 1998; Casillas *et al*., 1995, 1998). Similarly, Heinz *et al*. (1999) report increased mortality in pink salmon embryos exposed to oiled gravel with total PAH concentrations in the 3800–4600 ppb range. Consequently, an effect threshold is likely at or below this level. This raises some concern about whether some current sediment quality criteria for PAHs, which allow total PAH concentrations in the 20000 ppb range or above (WAC, 1995; USACE, 1998) are adequately protective of listed salmonids.

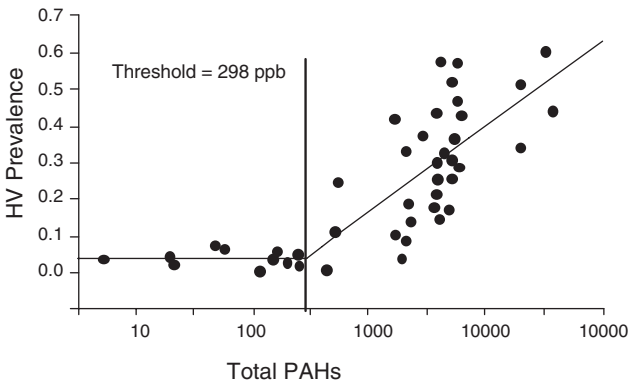


Figure 6. Hockey-stick regression of hydropic vacuolation (HV) prevalence in winter flounder versus total polycyclic aromatic hydrocarbons (TPAHs) in bottom sediment in ng/g dry wt. (ppb). Data were collected as part of NOAA's National Benthic Surveillance Program for the Northeast Coast of the United States (Johnson *et al*., 1992, 1993; Harmon *et al*., 1998). Threshold concentration is indicated by vertical line. Adapted from Lomax *et al*. (1994).

SEDIMENT QUALITY THRESHOLD GUIDANCE

In order to assess the likely degree of injury experienced by English sole exposed to sediments with different sediment PAH concentrations, expected prevalences of liver lesions and reproductive abnormalities were calculated using the regression equations from Table 2 for total PAH concentrations ranging from 50–100 000 ppb (ng dry wt.$^{-1}$). As illustrated in Table 3, liver lesion prevalences, as well as levels of other detrimental effects in English sole, were generally close to levels characteristic of fish from uncontaminated sites at sediment PAH concentrations below 1000 ppb. This concentration approximates the upper confidence levels of the thresholds for DNA damage and the occurrence of one or more hepatic lesions, and is close to the estimated threshold of 630 ppb for several reproductive effects. Above 1000 ppb, increases are observed in several categories of liver lesions, and reductions are observed in spawning ability and egg quality. On this weight of evidence basis, the sediment PAH threshold below which no significant carcinogenic or adverse reproductive effects in English sole are observed is estimated to be 1000 ppb (ng g^{-1} dry wt.), With sediment PAH concentrations at or below this level, sole should exhibit only minimal liver injury, and little or no disruption of growth or reproductive function, and relatively low levels of DNA

Table 3. Estimated effect levels associated with increasing sediment PAH concentration for selected liver lesions and indicators of reproductive function in English sole.

PAH (ppb dry wt.)	Neoplasm prevalence	FCA prevalence	SDN prevalence	Proliferative lesion prevalence	Any lesion prevalence
Liver lesions					
50	0.00	0.01	0.00	0.02	0.00
100	0.00	0.02	0.00	0.02	0.00
1000	**0.00**	**0.06**	**0.01**	**0.08**	**0.09**
2000	0.00	0.07	0.12	0.11	0.18
3000	0.01	0.08	0.20	0.13	0.24
5000	0.03	0.09	0.27	0.14	0.31
10000	0.06	0.10	0.38	0.17	0.40
100000	0.16	0.14	0.75	0.26	0.71

Reproductive indicators PAH (ppb dry wt.)	Inhibited gonadal development prevalence	Inhibited spawning prevalence	Infertile proportion eggs of eggs spawned	DNA Damage (nmol adducts per mol nucleotides)
50	0.15	0.12	0.38	5
100	0.15	0.12	0.38	5
1000	**0.15**	**0.17**	**0.42**	**25**
2000	0.15	0.25	0.48	36
3000	0.15	0.30	0.51	43
5000	0.18	0.35	0.55	51
10 000	0.27	0.43	0.61	63
100 000	0.58	0.69	0.80	100

For all liver lesions, inhibited gonadal development, and inhibited spawning, the effect level is the proportion of fish estimated to be affected at the indicated sediment PAH concentration; for infertile eggs, the effect level is the proportion of eggs produced by an individual female that are estimated to be unfertile. Effect levels for liver lesions were calculated with hockey-stick regression. For reproductive indicators, effect levels at the sampling sites where PAH concentrations were lowest were used to estimate background effect levels (i.e. effect levels at PAH concentrations below 5000 ppb for inhibited gonadal development, and below 1000 ppb for inhibited spawning and infertile eggs).

adducts. While this threshold is well below current sediment quality criteria for PAHs as set by the Washington State Department of Ecology (7400 ng g^{-1} for low molecular weight AHs, and 19 200 ng g^{-1} for high molecular weight AHs (WAC, 1995), assuming a sediment TOC content of 2%, a fairly typical value for Puget Sound sediment (Michelsen and Bragdon-Cook, 1993)), it is not overly conservative. Based on threshold values calculated for non-neoplastic liver lesions and DNA damage in English sole, a lower value could be justified. Moreover, the 1000 ng g^{-1} dry wt. guideline does not incorporate a safety factor, as risk analyses often do, to account for uncertainty in this threshold estimate due to factors such as variations in sensitivity of fish species, or PAH analytes measures, or in ratios of low to high molecular weight PAHs in sediments. We recommend the 1000 ppb threshold as a practical value for making management decisions, as it would be protective of estuarine fish populations, but not unworkable from the perspective of sediment remediation and management.

Above the threshold effects concentration of 1000 ppb, the proportion of animals affected and the number of adverse effects observed increases. The degree of increase is modeled by the upper arm of the hockey stick regression, which can be used to estimate the likely degree of injury at various sediment PAH concentrations (Table 3). At 5000 ppb, for example, levels of hepatic DNA adducts would be approximately 10-fold the levels found in fish from uncontaminated reference sites, about 30% of the population is predicted to have some form of liver disease, and the number of fish failing to spawn would increase from about 12% to over 35%. At PAH concentrations of 10 000 ppb, DNA adducts levels would have increased 12–13-fold, 50% of the sole would be expected to have liver disease, nearly 30% of the females would show inhibition of gonadal growth, and over 40% would show inhibition of spawning. This type of information, in combination with data on contaminant effects on other indigenous species, could be used to estimate the loss of productivity or ecosystem services due to PAH contamination at impacted sites.

CONCLUSIONS

Based on the analyses above, we determined threshold sediment PAH concentrations for toxicopathic liver lesions in English sole ranging from 54 to 2800 ng g^{-1} dry wt, and a threshold for DNA adducts in liver of 300 ng g^{-1} dry wt. Although we do not have sufficient data to statistically determine precise thresholds for other types of injury to English sole, available data indicate that several other types of impairment, including inhibited gonadal growth, inhibited spawning, reduced egg viability, and reduced growth, begin to occur at sediment PAH concentrations in a similar range. Based on these data, a sediment quality guideline of 1000 ppb total PAH (ng g^{-1} dry wt.) is suggested to protect estuarine fish against several important health effects, including selected degenerative liver lesions, spawning inhibition, and reduced egg viability. With sediment PAH concentrations at or below 1000 ppb, liver lesion prevalences, DNA adduct levels, and growth and reproductive indicators are generally similar to levels observed in English sole from reference sites with minimal sediment PAH contamination, and English sole exhibit little or no toxicopathic injury. Above 1000 ppb, there appears to be a substantial increase in the risk of liver disease and reproductive impairment, as well as potential effects on growth. As sediment PAH concentrations increase, the proportion of animals affected and the number of adverse effects observed steadily increases. These relationships can be used to help assess the likely degree of injury to marine resources at various sediment PAH concentrations.

Unfortunately, achieving PAH thresholds in estuarine sediments in the United States would be a substantial task, as sediment contamination at this level is widespread. For example, in a recent EPA report on contaminated sediments in the Untied States, sediments from nearly 6000 stations throughout the country were examined for PAHs, and of these 36% were within the range where adverse effects were considered probable or possible, with concentrations exceeding the 1000 ppb recommended threshold (USEPA, 1997). Moreover, active sources of PAH input, such as urban runoff and atmospheric deposition,

continue to contribute to environmental releases. However, our data suggest that failure to achieve such standards will result in impaired productivity of our fish stocks, which, in combination with other stressors, such as overharvest and habitat destruction, could put additional species in danger of extinction.

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